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Measles Virus RNA Is Not Detected in Inflammatory Bowel Disease Using Hybrid Capture and Reverse Transcription Followed by the Polymerase Chain Reaction

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Recent epidemiological and immunohistochemical studies have indicated a possible link between measles virus and inflammatory bowel disease (IBD). The aim of this study was to use a sensitive and robust method for the detection of measles virus RNA in IBD and control clinical samples.

Peripheral blood mononuclear cells and intestinal resection tissue from IBD and control patients were studied. Two methods were used to determine the presence of measles virus RNA: hybrid capture, using measles virus-specific oligonucleotides linked to paramagnetic solid-phase supports, was carried out on total cellular RNA to enrich for measles virus RNA sequences. Reverse transcription followed by the polymerase chain reaction (RT-PCR) using rTth DNA polymerase was employed for amplification of measles virus N-gene sequences amongst the enriched species. Total RNA was also used for RT-PCR of a housekeeping mRNA species to assess RNA quality. RT-PCR for another region of the measles genome (the haemagglutinin (H) gene) was also undertaken in order to confirm the results obtained using N-gene primers for analysis of these samples.

None of the samples were positive for measles N- or H-gene RNA using RT-PCR. Positive control samples confirmed the sensitivity of the methods employed. These results show that either measles virus RNA was not present in the samples, or was present below the sensitivity limits known to have been achieved. ***J. Med. Virol. 55:305–311, 1998.***

KEY WORDS: Crohn's disease; ulcerative colitis; RNA amplification

INTRODUCTION

Crohn's disease is a chronic inflammatory bowel disorder of unknown aetiology that is characterised histologically by the presence of submucosal granulomas. Granulomatous vasculitis has been proposed as an early event in the pathogenesis of Crohn's disease, leading to inflammation and ischemia [Wakefield et al., 1991], although the origin of these granulomas remains unclear. One hypothesis is that granulomatous vasculitis can result from an immune reaction against a persistent measles virus infection of the submucosal endothelium [Wakefield et al., 1995]. It has been observed that measles virus can persist in endothelial cells of the central nervous system (CNS) [Kirk et al., 1991] where it is known to be associated with the degenerative CNS diseases, subacute sclerosing panencephalitis (SSPE), and measles inclusion body encephalitis (MIBE).

Several epidemiological studies have implicated early exposure to measles virus [Ekbom et al., 1994, 1996] or the live attenuated measles vaccine [Thompson et al., 1995] in the aetiology of Crohn's disease. In fact, in situ hybridisation and immunohistochemistry have shown the presence of measles virus RNA [Wakefield et al., 1993] and measles virus nucleoprotein [Wakefield et al., 1993, 1997], respectively, in the foci of submucosal granulomas in Crohn's disease. Furthermore, immunogold electron microscopy has shown the presence of very low copy-number measles virus nucleocapsids in the foci of submucosal granulomas in the majority of cases of Crohn's disease [Lewin et al., 1995; Ekbom et al., 1996]. However, other groups have failed

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to detect measles virus RNA in clinical samples from Crohn's disease patients using RT-PCR [Iizuka et al., 1995; Lewey et al., 1995; Fan et al., 1996; Haga et al., 1996]. In these studies, the methods employed for the amplification of measles virus RNA may not have been sufficiently sensitive to permit the detection of low copy-number measles virus RNA molecules, which may have been present [Chadwick and Wakefield, 1996].

In this study, measles virus-specific hybrid capture, prior to amplification reactions, was used as described previously [Chadwick et al., 1997] to enrich for any measles virus RNA in total cellular RNA isolated from IBD and control tissue samples. The enrichment for measles RNA using hybrid capture prior to RT-PCR was found to increase the sensitivity of measles RNA detection 100-fold compared to RT-PCR alone.

A transcription gradient from the 3′ end (containing the nucleoprotein [N] gene) to the 5′ end (containing the large [L] gene) of the genome is characteristic of measles virus replication and this gradient often is exaggerated in persistent infections [Sidhu et al., 1994]. Consequently, the measles virus nucleoprotein gene sequence was selected as a target for amplification in this study. To obtain a measure of the sensitivity of our methods, a serial dilution of a wild-type (wt) measles virus nucleoprotein RNA transcript was also used as a target for amplification. A modified measles virus RNA transcript, yielding a larger product than the wt, was used to monitor reaction efficiency [Chadwick et al., 1997]. Both transcripts were derived originally from the measles virus Hu2 strain [Taylor et al., 1991]. The RNA sequence of the measles Hu2 strain is known and amplification products from positive samples can be sequenced in order to eliminate the possibility of false positive results due to PCR contamination. Moreover, a region of sequence diversity is present between the PCR primer binding sites permitting several different strains of measles virus to be distinguished [Taylor et al., 1991].

Persistent infection of PBMCs by measles virus has been shown in a variety of diseases. For instance, Kawashima et al. [1996] recently demonstrated the presence of measles virus H-gene RNA in the PBMCs of patients with autoimmune hepatitis. In view of this, PBMCs (in addition to intestinal resection tissue) from IBD and control patients were analysed for the presence of measles virus N- and H-gene RNA.

The purpose of this study was to use a sensitive and robust method to analyse clinical samples from patients with inflammatory bowel disease and controls for the presence of measles virus RNA.

TABLE I. Details of Patients in Study*

Patient	Disease	Age yrs	Sex m/f
Intestinal tissue			
1	Crohn's	25	M
2	UC	51	M
3	Sigmoid volulus	60	M
4	Crohn's	22	F
5	Crohn's	36	F
6	Crohn's	17	F
7	UC	55	M
8	Crohn's	41	M
9	UC	52	F
10	UC	58	M
11	Ischaemia	49	F
12	Crohn's	36	F
13	Carcinoma	78	F
14	Carcinoma	68	F
15	Crohn's	40	M
16	Crohn's	27	M
17	Carcinoma	52	M
18	Carcinoma	45	F
19	Crohn's	21	F
20	Crohn's	37	F
Measles-infected tissue			
81	SSPE	21	M
83	SSPE	11	M
214	SSPE	14	M
300	SSPE	6	M
Peripheral blood cells			
A	Indeterminate colitis	45	F
B	UC	74	F
C	Crohn's	50	M
D	Crohn's	25	F
E	Crohn's	55	F
F	Crohn's	34	M
G	Indeterminate colitis	41	F
H	Crohn's	49	M
I	Indeterminate colitis	13	F
J	Rectal bleed	8	M
K	UC	14	M
L	Crohn's	16	M
M	Autoimmune heppatitis	32	F
Positive control PBMCs			
X	Vaccine recipient	NA	NA
Y	SSPE	NA	M
Z	SSPE	NA	M

*All Crohn's disease tissue contained granulomas. NA, Not available.

MATERIALS AND METHODS

RNA Transcripts

pT7Blue E Transcript (wt transcript). The 1198-1631 nt region of the measles virus genome was cloned into the pT7Blue vector (Novagen, AMS Biotechnology, Witney, UK), and the product linearised to transcribe a 508 nt RNA in vitro [Chadwick et al., 1997]. This transcript represented a genomic (−) measles virus RNA sequence and contained the primer binding sites subsequently used in measles virus RNA detection experiments. Its amplification generated a 253 bp product (see Table I for primer details).

pT7Blue E144 Transcript (modified transcript). A modified internal control transcript was produced as described previously [Chadwick et al., 1997]. Briefly, a 144 bp DNA fragment was cloned into the Nsi I restriction site of the measles virus cDNA insert in pT7Blue, between the PCR primer binding sites. The plasmid was linearised and in vitro transcription was used to produce the modified transcript. RT-PCR using this modified transcript yielded a 386 bp product.

Tissue Samples

All IBD tissue was derived from patients with end-stage disease undergoing intestinal resection. Diseased IBD tissue (based on macroscopic analysis) was used for study, while macroscopically normal tissue was taken for study from resection margins of tissue from control patients. Approximately 500 mg fresh-frozen intestinal resection tissue was analysed from Crohn's disease (n = 10), ulcerative colitis (n = 4), and control patients undergoing resection for intestinal carcinoma (n = 6). Postresection time prior to snap-freezing in liquid nitrogen was less than 5 min. for all cases and tissue was stored at !70EC until RNA extraction for up to 5 years.

On histological examination, all IBD tissues showed evidence of inflammation. Previous studies have shown immunohistochemical evidence of persistent measles virus in the foci of Crohn's disease granulomas [Wakefield et al., 1993, 1997; Lewin et al., 1995; Ekbom et al., 1996]. With this in mind, all Crohn's disease tissues were selected on the basis that they contained granulomas. Patient details are shown in Table I.

Blood Samples

Blood samples were taken from patients with Crohn's disease (n = 6), indeterminate colitis (n = 3), ulcerative colitis (n = 2), and control patients (n = 2) following outpatient appointments. PBMCs were immediately isolated from 10 ml of blood (taken in ethylenediaminetetra-acetic acid [EDTA] tubes) on a ficoll density gradient (Lymphoprep™, Nycomed Pharma AS, Oslo, Norway) according to the manufacturers' instructions. Cells were washed in phosphate-buffered saline (PBS), pelleted and stored at −70°C until RNA extraction. Patient details are shown in Table I.

RNA Extraction

Total RNA was extracted from coded intestinal tissues or PBMCs using a standard acid guanidinium phenol-chloroform method [Chomczynski and Sacchi, 1987]. Resection specimens were homogenised in the appropriate buffer using a T25 Ultra Turrex with autoclaved dispersing shaft (Marathon, London, UK) at 0°C and PBMCs were lysed in the same. Tissue homogenates were spiked with 107 copies of modified transcript as a positive internal control to determine the possible negative effects of RNA isolation on subsequent measles RNA amplification. RNA pellets were washed twice with 70% ethanol, resuspended in 100 µl of water and stored at −70°C.

One µg of total isolated RNA was analysed by agarose gel electrophoresis for the presence of RNA degradation.

Hybrid Capture for Measles Virus N-Gene RNA

Oligonucleotides HC1 and HC2 (Table I) were synthesised (University of Greenwich, London, UK) and linked to magarose beads (Whatman plc, Maidstone, UK) as described previously [Bruce et al., 1996]. Derivatised magarose was washed three times with 2× binding/washing buffer (containing 10 mM Tris pH 7.5, 1 mM EDTA, 2 mM NaCl) at 20°C for 5 min prior to the addition of 50 µl of intestinal RNA and 50 µl of binding/washing buffer. After incubation of the mixture at 20°C for 10 min. with gentle agitation, the supports were immobilised magnetically and the supernatant removed. Supports were washed three times at 20°C for 5 min with binding/washing buffer and captured RNA eluted in 50 µl of water by heating to 65°C for 10 min with gentle agitation. Hybrid capture eluents were stored at −70°C. Hybrid capture was not carried out on PBMC RNA samples.

U1A RNA RT-PCR

RT-PCR for a housekeeping RNA species, U1A (comprising part of the spliceosome complex [Sillekens et al., 1987]) was carried out to assess the efficiency of isolation of undegraded RNA. Primers U1A1 and U1A2 (Table II; Perkin Elmer, Warrington, UK) were used together with 100 ng of total sample RNA (without hybrid capture) in a combined RT-PCR reaction using rTth DNA polymerase and EZ buffer (Perkin Elmer) according to the manufacturers' instructions. Amplification was carried out using the following cycling parameters: Reaction mixtures were placed in a Techne PHC3 thermal cycler (Techne, Cambridge UK) and incubated at 60°C for 30 min followed by denaturation at 95°C for 2 min. They were then subjected to 40 cycles of 95°C for 1 min and 58°C for 1 min. After a final extension of 60°C for 7 min, PCR products were electrophoresed in a 1.2% agarose gel and visualised by staining with ethidium bromide and exposure to u.v. light. Products were tranferred subsequently to a Hybond-N membrane (Amersham, Bucks., UK) according to the manufacturers' instructions. To permit confirmation of product specificity, hybridisation was performed using a ^{32}P-labelled oligonucleotide probe U1A (Table II), internal to the region amplified.

Measles Virus Nucleoprotein Gene RT-PCR

Combined RT-PCR reactions, using rTth DNA polymerase and EZ buffer, and containing 5 µl of hybrid capture elutants were carried out according to the manufacturers' instructions, using primers MV3 and MV4 (Table II). Reaction mixtures were cycled thermally and products analysed using a ^{32}P-labelled oligonucleotide AB10 (Table II) internal to the product as described above.

Measles Virus Haemagglutinin Gene RT-PCR

H-gene RT-PCR was carried out on all total RNA samples (without prior hybrid capture) using a method identical to that of Kawashima et. al. [1996]. Primer MF1 was used for reverse transcription and primer pairs M3/H6 and H4A/H7 (Perkin Elmer) were used for

TABLE II. The Sequences and Positions of the U1A, Measles N-Gene, and Measles H-Gene Amplification Primers and Probes*

Primer	Position	Sequence
U1A1 (Downstream)	583–603	GCC CCG GCA TGT GGT GCA TAA
U1A2 (Upstream)	378–400	CAG TAT GCC AAG ACC GAC TCA GA
U1A (Probe)	451–472	AGA AGA GGA AGC CCA AGA GCC A
MV3 (Upstream)	1248–1269 (N-gene)	AGC ATC TGA ACT CGG TAT CAC
MV4 (Downstream)	1480–1501 (N-gene)	AGC TCT CGC ATC ACT TGC TCT
MV5 (Downstream)	1348–1368 (N-gene)	AGA AAT GAT ACT TGG GCT TGT
AB10 (Probe)	1288–1308 (N-gene)	GTT TCA GAG ATT GCA ATG CA
MF1 (Upstream)	7209–7233 (F-gene)	GCT TCC CTC TGG CCG AAC AAT ATC G
M3 (Upstream/Outer)	8106–8125 (H-gene)	CAG TCA GTA ATG ATC TCA GC
6 (Downstream/Outer)	8677–8701 (H-gene)	CTT GAA TCT CGG TAT CCA CTC CAA T
H7 (Upstream/Inner)	8147–8171 (H-gene)	GAG CTC AAA CTC GCA GCC CTT TGT C
H4A (Downstream/Inner)	8458–8482 (H-gene)	ATC CTT CAA TGG TGC CCA CTC GGG A
H5 (Probe)	8370–8392 (H-gene)	TCC CGA CAA CAC GAA CAG ATG AC
HC1 (Downstream)	1328–1368 (N-gene)	AGA AAT GAT ACT TGG GCT TGT CTG GGT CCA ACC GCT CAT C
HC2 (Upstream)	1288–1322 (N-gene)	GTT TCA GAG ATT GCA ATG CAAC TAC TGA GGA CAA GAT CAG

*In addition, the measles N-gene hybrid capture sequences (HC1 and HC2) and positions are given.

first and second round PCR, respectively. Primer details are given in Table II. Second round PCR products were analysed as described above using a ^{32}P-labelled oligonucleotide probe H5 (Table II), internal to the PCR product.

Sequencing of Amplification Products

In order to generate enough PCR products for direct sequencing measles virus N-gene amplification products from rTth RT-PCR reactions were electrophoresed on a low melting temperature agarose gel, DNA of the correct size extracted using a PCR gel extraction kit (Promega, Southampton, UK), and PCR carried out using primers MV3 and MV5 (Table II) using the parameters described above. These products, as well as H-gene products, were sequenced directly (Microchemical Facility, Babraham Institute, Cambridge, UK).

Controls

Tissue containing a persistent measles virus infection consisted of fresh-frozen postmortem brain samples from four patients with SSPE (details given in Table I). The presence of measles virus in these tissues was confirmed by immunohistochemistry (data not shown).

Positive control PBMCs for measles virus consisted of RNA from a measles vaccine (strain AIK-C) recipient in Japan (gift from H. Kawashima, Tokyo, Japan) and PBMCs from two patients with SSPE (details given in Table I).

RESULTS

Positive Controls and Detection Sensitivity

The detection sensitivity limit for the detection of a wt measles RNA transcript using hybrid capture followed by RT-PCR has been described previously [Chadwick et al., 1997]. As few as 10^4 transcript molecules spiked into homogenates of intestinal tissue could be detected using this method. This represented a 100-fold increase in detection sensitivity compared to RT-PCR alone.

For the detection of measles RNA in PBMC samples, 10^4 molecules of wt measles transcripts could be detected in PBMC homogenates using RT-PCR without prior hybrid capture (data not shown). Moreover, the use of hybrid capture did not increase the detection sensitivity for wt measles RNA transcripts spiked PBMC lysates, therefore hybrid capture was not used to enrich total PBMC RNA for any measles RNA present (data not shown).

All four SSPE brain tissue samples (described in Table I) gave positive results by RT-PCR alone for measles virus N- and H-gene RNA (Fig. 1) and sequence analysis of amplification products showed that these had not arisen as a result of PCR contamination. However, the measles vaccine recipient PBMC RNA

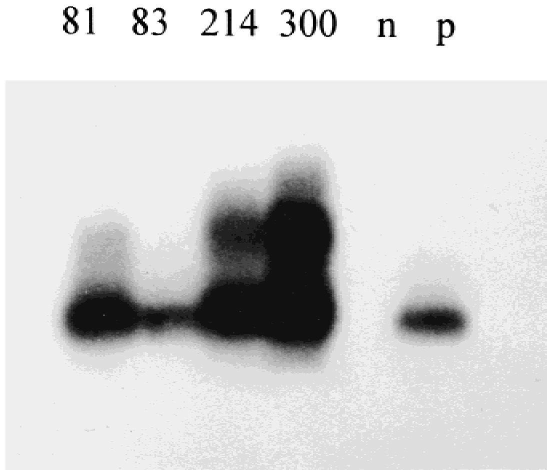

Fig. 1. Southern blot analysis of measles N-gene PCR products probed with ^{32}P-labelled internal oligonucleotide (AB10). 100 ng total RNA isolated from four autopsy brain tissue samples of SSPE patients (patients 81, 83, 214, and 300) was used for measles N-gene RT-PCR. Ten µl of PCR product was used for agorose gel electrophoresis, Southern blotting, and hybridisation. Reaction n = RT-PCR negative control containing water. Reaction p = RT-PCR positive control containing 100 ng total RNA from measles-infected Vero cells.

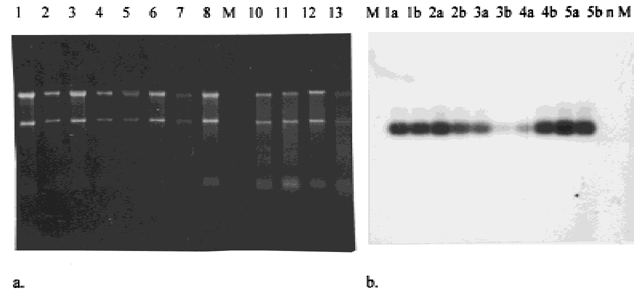

Fig. 2. **a:** Representitive agarose gel containing 1 µg total RNA isolated from PCMC pellets (lanes 1–8). Lanes 10–13 contain 1 µg total RNA from SSPE brain tissue (from patients 81, 83, 214, and 300). Lane M contains a 0.24–9.5 kb RNA size marker (Gibco, Paisley UK). **b:** Representative autoradiograph of U1A PCR products probed with a ^{32}P-labelled internal oligonucleotide (U1A). One hundred ng of total sample RNA was used for U1A RT-PCR (lanes 1a–5b) and 10 µl of PCR products used for agarose gel electrophoresis, Southern blotting, and hybridisation. Reaction n = RT-PCR negative control containing water. Lane M contains a 100 bp DNA size marker.

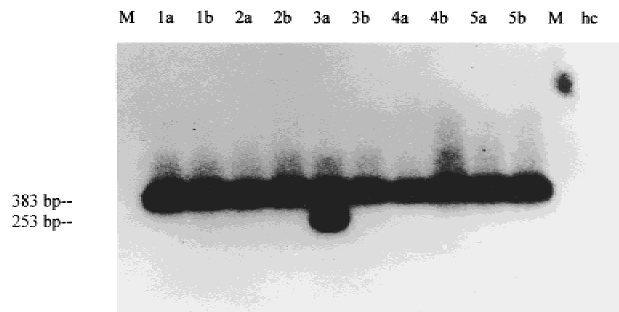

Fig. 3. Representitive Southern blot analysis of measles N-gene PCR products probed with a ^{32}P-labelled internal oligonucleotide (AB10). Duplicate total intestinal RNA samples **(a,b)** were used for hybrid capture and subsequent RT-PCR for measles N-gene RNA. Ten µl of PCR product was used for agorose gel electrophoresis, Southern blotting and hybridisation. The 383 bp band represents the PCR product from the modified measles internal transcript and the 253 bp band represents the PCR product from wt measles RNA. Lane M contains a 100 bp DNA size marker. Reaction Ahc contains a hybrid capture elutant using water as a negative control.

sample was found to be positive for the measles H-gene but negative for the measles N-gene, although the small amount of RNA available did not permit this experiment to be repeated and the results confirmed. Sequencing experiments confirmed the vaccine recipient virus to be the AIK-C strain. PBMC RNA from the two SSPE cases were negative for measles N- and H-genes.

RNA Sample Integrity

Ribosomal RNA bands (18S and 28S) could be clearly seen following agarose gel electrophoresis of isolated total RNA, indicating minimal RNA degradation (Fig. 2). Extracted RNA was found to be positive for U1A RNA in all but three cases, indicating successful extraction of high quality total RNA (Fig. 2). Internal modified transcripts were amplified from extracted intestinal RNA in all cases, implying that the RNA extraction protocol had not been destructive to measles virus RNA (Fig. 3).

Measles RNA

Two intestinal RNA samples and one PBMC RNA sample gave positive signals following amplification, Southern blotting, and hybridisation for measles virus N-gene PCR products. All other samples gave negative results for measles N-gene RNA. An example of a positive signal from intestinal tissue is shown in Figure 3. All samples analysed gave negative results for measles H-gene RNA following amplification, Southern blotting, and hybridisation.

Sequencing of these products showed that the wt measles N-gene PCR products possessed an identical sequence to that of the laboratory-strain positive control virus (Hu2), indicating that these PCR products almost certainly arose as a result of PCR contamination. Confirmation was by virtue of a guanidine substitution for adenine at position 1336 of the measles virus genome. Moreover, for each positive sample, the duplicate RNA sample was negative for measles RNA and no sample was positive for measles H-gene RNA, further indicating that the positive measles N-gene results were artifactual.

DISCUSSION

Measles virus RNA was not detected in PBMCs of patients with SSPE, in agreement with the results of Schneider-Schaulies et al. [1991]. This could have been due to two reasons: measles virus RNA could have been present below the sensitivity limits described; or persistent measles virus may not have been present in the peripheral blood of the SSPE patients. Contrary to these data, measles virus H- and N-gene RNA was identified in brain tissue from all four patients with SSPE, confirming the ability of the methods described

to detect persistent measles virus RNA in clinical samples.

Hybrid capture did not improve the detection sensitivity of measles RNA in PBMC lysates because the amount of PBMC RNA generated was too small for further enrichment. Therefore hybrid capture was not employed for the detection of measles RNA in PBMC samples.

Total RNA samples were of sufficient quality, as assessed by RT-PCR for a low copy-number housekeeping RNA species (U1A RNA). Spiking experiments have shown the measles virus RNA detection method described here to be sufficiently sensitive to detect as few as 10^4 molecules [Chadwick et al., 1997]. Lund et al. [1984] demonstrated that up to 13,000 nucleocapsids are present per functional measles virion. As each nucleocapsid contains one molecule of genomic measles RNA, then the detection of 10^4 measles RNA molecules represents the detection of as few as ten functional measles virions. Moreover, the measles RNA detection assay described here is capable of detecting genomic, antigenomic, and messanger measles RNA, further emphasizing the sensitivity of this method.

Other than as a contaminant, measles virus RNA was not detected in any samples from IBD or control patients in the experiments reported in this investigation, supporting results from other laboratories [Iizuka et al., 1995; Lewey et al., 1995; Fan et al. 1996; Haga et al., 1996]. The presence of PCR contamination could be unequivocally demonstrated by the sequencing of measles N-gene PCR products and analysis for a guanidine substitution of adenine at position 1336—characteristic of the measles laboratory Hu2 strain sequence. Therefore, although false positive results were generated, they were not misinterpreted as resulting from measles virus-infected clinical samples.

Kawashima et al. [1996] have been able recently to detect wt and vaccine strains of measles virus H-gene RNA in PBMCs of patients with autoimmune hepatitis. In this study however, no measles virus RNA was detected in PBMCs from IBD or control patients except from PBMC RNA from a vaccine recipient which was only positive for amplification of measles virus H-gene RNA. The inability to detect measles virus N-gene RNA from a clinical positive control sample may, however, reflect specific problems associated with the extraction of measles virus N-gene RNA observed by others [D. Griffin, Johns Hopkins University, Baltimore, MD; personal communication]. For instance, strong binding between measles virus nucleoprotein and N-gene RNA, as reported by other groups [Andzhaparidze et al., 1987; Moyer et al., 1990], may inhibit the efficient isolation and amplification of measles RNA in clinical samples, possibly accounting for the negative results described here.

The negative PCR results reported in this study indicate that either measles RNA was not present in the samples tested, or was present below the limits of sensitivity. If measles RNA was not present in the Crohn's disease tissue analysed, then the results of immunohistochemistry studies for measles antigens in IBD [Wakefield et al., 1993, 1997; Lewin et al., 1995; Ekbom et al., 1996] must be re-examined, especially in the light of a recent report concerning the specificity of the measles monoclonal antibody used in the original study [Iisuka and Masamune, 1997]. It is possible that the measles antigen immune staining methodologies gave artifactual results in these studies.

Immunogold electron microscopy data has suggested the presence of measles virus in the foci of Crohn's disease granulomas [Lewin et al., 1995]. If the number of copies of measles RNA is less than 10^4 per sample (i.e., below the sensitivity limit of the detection method), then it may be possible to enrich for measles virus RNA by extracting granulomatous areas from either frozen or paraffin-embedded Crohn's disease tissue. An alternative could be the co-cultivation of PBMCs with African green monkey kidney Vero cells which may permit the replication of any measles virus, and therefore enhancement of any measles RNA present. Also, it has been possible recently to culture human intestinal microvascular endothelial cells from Crohn's disease resection tissue [Haraldsen et al., 1995]. Analysis of RNA from these cells may increase the possibility of detecting a measles virus infection of the submucosal endothelium. Such studies are currently underway in our laboratory.

The apparent disparity between negative PCR and positive immunogold results may also be explained by the presence of viral antigens, but not RNA in IBD intestinal tissue. It has recently been postulated that a mechanism for the longevity of the immune response to measles virus could involve persistence of measles virus antigens but not RNA, as follicular dendritic cells in lymphoid tissue may retain measles antigens for long periods following a measles virus infection and possibly measles vaccination [Griffin et al., 1994]. In this way, memory B cells may be stimulated, conferring long-term immunity. This mechanism could explain the detection of measles nucleoprotein in the intestinal tissue of patients with Crohn's disease by various methods [Lewin et al., 1995; Wakefield et al., 1993], without the detection of measles RNA as described here.

A recent publication by Klenerman et al. [1997] has identified lymphocytic choriomeningitis virus (LCMV) DNA in tissue from infected mice. This finding is highly significant since LCMV (like measles virus) is a nonretroviral RNA virus. The authors described the presence of LCMV antigen and DNA in the absence of LCMV RNA, suggesting that LCMV DNA may be a natural form of DNA vaccine, periodically producing transient amounts of viral antigen to stimulate the immune memory. The detection of LCMV DNA and antigens in the absence of LCMV RNA may be important in the context of the findings of measles nucleoprotein in Crohn's disease tissue in the apparent absence of measles RNA described in this study. Apart from one early study [Zdanov, 1975] the possibility that measles virus may persist in a DNA form has not been investi-

gated. Experiments are underway in our laboratory to investigate this possibility and to determine its relevance in the context of IBD.

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